ScdA: Unveiling the Nitrite-Reducing Machinery That Helps *Staphylococcus aureus* Survive

A tri-disciplinary team uncovered how the elusive ScdA protein in Staphylococcus aureus forms a dimeric structure to generate nitric oxide, revealing a new mechanism of bacterial stress resistance.

For nearly two decades, the enigmatic di-iron protein ScdA of *Staphylococcus aureus (S. aureus)* puzzled microbiologists. Identified in 2008 and grouped within the "repair of iron centers" family, ScdA was believed to protect the bacterium's iron-sulfur enzymes from oxidative and nitrosative damage.[1] Yet, despite its presumed importance in the pathogen's defense against immune attack, no one had ever visualized the protein's complete structure, or fully understood what it actually did.

A recent study published in the *Journal of the American Chemical Society* (2025) answers both questions.[2] The researchers revealed that ScdA is not merely a repair enzyme, but it is a nitrite reductase that transforms nitrite (NO_2^-) into nitric oxide (NO), a reactive molecule with profound biological consequences. By combining X-ray crystallography, solution-state nuclear magnetic resonance (NMR), AlphaFold modeling, and pulsed electron spin resonance (ESR)/double electron–electron resonance (DEER) spectroscopy, they reconstructed the first full-length structure of dimeric ScdA and linked its architecture directly to its catalytic function.

A Long-Standing Mystery in Bacterial Stress Biology

S. aureus—a major human pathogen and the source of methicillin-resistant infections (MRSA)—endures some of the harshest chemical assaults mounted by the immune system. Host macrophages generate reactive oxygen and nitrogen species to damage bacterial macromolecules. ScdA is expressed as a stress response, regulated by the two-component system SrrAB. Although earlier studies hinted that ScdA repaired damaged iron-sulfur clusters, the details of its structure and mechanism of action remained elusive.

The central question persisted: Was ScdA merely a repair enzyme, or did it play a more active role in managing nitrosative stress?

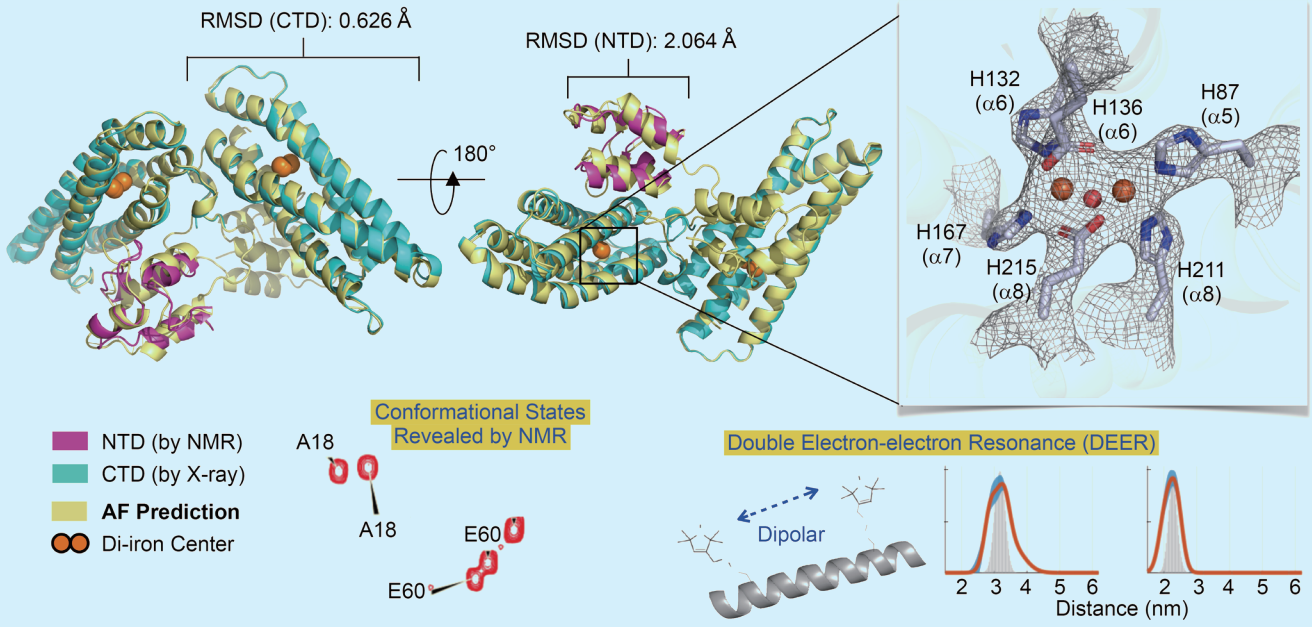

Fig. 1: Integrative structural model of full-length ScdA constructed from X-ray crystallography, NMR spectroscopy, and DEER distance measurements. The CTD di-iron domain (cyan) was resolved by X-ray diffraction at the NSRRC, the NTD domain (magenta) by NMR, and the composite model (yellow) refined with AlphaFold and DEER constraints. The di-iron catalytic center (orange spheres) and coordinating residues are shown in electron density maps. RMSD values indicate high structural convergence for the CTD (0.626 Å) and moderate flexibility in the NTD (2.064 Å). DEER-derived distance distributions and spin-labeling schematics demonstrate how pulsed ESR measurements define domain orientations and validate the dimeric architecture of ScdA. [Reproduced from Ref. 2]

To answer this, the researchers needed not only high-resolution structural data but also insight into how the protein behaves in solution and during catalysis. Solving the full-length structure of ScdA required three complementary approaches and an exceptional collaboration across institutions.

Seeing the Whole Picture: A Three-Way Collaboration

The C-terminal domain (CTD) of ScdA, which contains the di-iron catalytic center, was the first piece to fall into place. After more than three years of work, the team obtained crystals suitable for X-ray diffraction and collected crystallographic data at **TPS 05A** in the NSRRC. The resulting structure revealed a compact four-helix bundle containing six conserved residues—histidines and glutamates—that coordinate the two iron atoms (**Fig. 1**).

The N-terminal domain (NTD), by contrast, refused to crystallize. Using high-field NMR spectroscopy, they determined that this flexible region adopts a smaller helical fold. To bridge these two structural fragments, the team employed AlphaFold-3 predictions to generate a full-length model and validated it experimentally using DEER spectroscopy (**Fig. 1**).

DEER, an advanced pulsed ESR technique, measures nanometer-scale distances between spin labels placed on the protein surface.[3,4] Using site-directed spin labeling of ScdA, they mapped inter-residue distances across both domains, confirming that ScdA exists as a homodimer in solution. The DEER-derived distance distributions closely matched those predicted by AlphaFold, establishing the model as a realistic representation of ScdA's dynamic structure.

This integrative approach—X-ray, NMR, and DEER—was possible only through the collaboration of three research groups: Yun-Wei Chiang (ESR/DEER, National Tsing Hua University), Shih-Che Sue (NMR, National Tsing Hua University), and Nien-Jen Hu (X-ray crystallography, National Chung Hsing University). Each method resolved a different aspect of ScdA's architecture; together they revealed the complete molecular geometry (**Fig. 1**).

From Structure to Function: ScdA Generates Nitric Oxide

With the structure in hand, the researchers turned to investigate function. Spectroscopic and kinetic experiments showed that ScdA catalyzes the reduction of nitrite to nitric oxide at its di-iron center. Ultraviolet–visible spectra revealed the formation of an iron–nitrosyl complex upon nitrite addition, while low-temperature EPR spectra displayed distinct signatures of mononitrosyl and dinitrosyl intermediates.

Kinetic experiments indicated robust nitrite-to-NO turnover comparable to other bacterial nitrite reductases. Interestingly, ScdA's dimeric form exhibited higher catalytic efficiency than its monomeric variants, suggesting that dimerization tunes electron transfer and substrate binding. Mutating a key interface residue (Ser77) disrupted dimer formation and reduced enzymatic performance, underscoring the functional importance of the dimeric assembly (**Fig. 2**, see next page).

Nitric Oxide: A Double-Edged Sword

Nitric oxide is a paradoxical molecule—both a weapon and a hazard. In the immune system, it serves as an antimicrobial agent, yet at controlled levels, it also acts as a signaling molecule. In-cell assays revealed that *Escherichia coli* engineered to overexpress ScdA suffered severe growth inhibition in nitrite-rich environments, demonstrating the cytotoxic potential of ScdA-generated NO.

In *S. aureus*, however, ScdA's activity is likely tightly regulated, enabling the bacterium to harness small, localized bursts of NO to modulate its redox balance without succumbing to toxicity. Understanding how this regulation operates could open new routes to antimicrobial intervention by destabilizing this balance, researchers may be able to sensitize MRSA and related pathogens to immune defenses.

A Technological and Biological Milestone

Beyond its biological insights, the study showcases the power of integrative structural biology. Advanced pulsed ESR/DEER spectroscopy provided the crucial link that unified data from X-ray and NMR studies, demonstrating how modern ESR can visualize entire protein architectures that are otherwise inaccessible. The approach offers a general blueprint for mapping large, multi-domain complexes whose flexibility frustrates traditional crystallography.

From a societal standpoint, the implications reach far beyond one bacterial enzyme. Understanding how pathogens like *S. aureus* regulate reactive nitrogen chemistry could inform strategies for next-generation antibiotics or redox-modulating

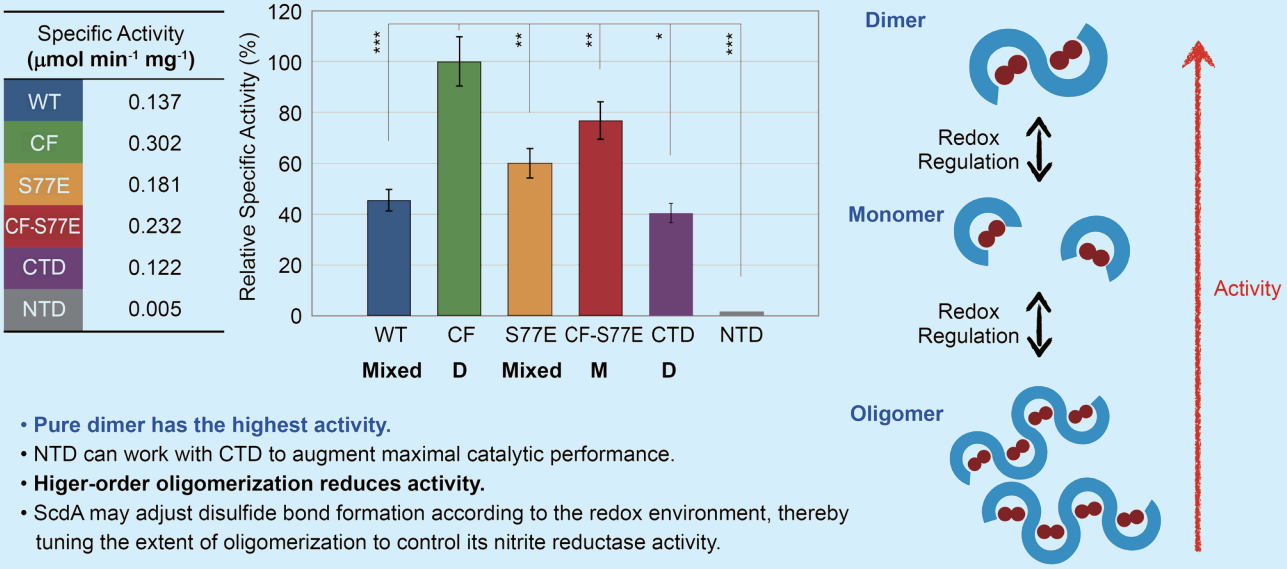

Fig. 2: Redox-dependent oligomerization governs the catalytic efficiency of ScdA. The bar chart compares nitrite reductase activities of wild-type and mutant variants. The cysteine-free (CF) ScdA, which forms a stable dimer, exhibits the highest activity by avoiding disulfide-linked oligomerization that otherwise suppresses catalysis. The S77E mutation, located at the dimer interface, disrupts dimer formation and stabilizes the disulfide-linked oligomeric state, resulting in reduced activity. CF-S77E stabilizes the monomeric state. Schematics on the right illustrate how redox regulation modulates transitions among oligomeric, dimeric, and monomeric forms, thereby fine-tuning enzymatic output. These findings indicate that ScdA adjusts its disulfide bonding and oligomerization state in response to redox conditions to optimize nitrite reductase activity. [Reproduced from Ref. 2]

therapies. ScdA—once an obscure stress-response protein—now emerges as a key player in bacterial survival and a potential target for antimicrobial design. (Reported by Nien-Jen Hu, National Chung Hsing University; Shih-Che Sue and Yun-Wei Chiang, National Tsing Hua University)

This report features the work of Yun-Wei Chiang and his collaborators published in J. Am. Chem. Soc. ***147****, 31558 (2025).*

TPS 05A Protein Microcrystallography

- Protein Crystallography
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References

1. T. W. Overton, M. C. Justino, Y. Li, J. M. Baptista, A. M. P. Melo, J. A. Cole, L. M. Saraiva, J. Bacteriol. **190**, 2004 (2008).
2. H.-Y. Chen, R.-F. Tsai, Y.-S. Lu, Y.-C. Cheng, H.-Y. Fan-Chiang, C.-Y. Wu, F.-C. Lo, H.-W. Kuo, W.-K. Yang, W.-Y. Liao, N.-J. Hu, S.-C. Sue, Y.-W Chiang, J. Am. Chem. Soc. **147**, 31558 (2025).
3. P.-S. Yeh, C.-C. Li, Y.-S. Lu, Y.-W. Chiang, JACS Au **3**, 2918 (2023).
4. C.-C. Cheng, R.-F. Tsai, C.-K. Lin; K.-T. Tan, V. Kalendra, M. Simenas, C.-W. Lin, Y.-W Chiang, JACS Au **4**, 3766 (2024).